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REVIEW

The functions of TRPA1 and TRPV1: moving away from sensory nerves

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The transient receptor potential vanilloid 1 and ankyrin 1 (TRPV1 and TRPA1, respectively) channels are members of the TRP superfamily of structurally related, non-selective cation channels. It is rapidly becoming clear that the functions of TRPV1 and TRPA1 interlink with each other to a considerable extent. This is especially clear in relation to pain and neurogenic inflammation where TRPV1 is coexpressed on the vast majority of TRPA1-expressing sensory nerves and both integrate a variety of noxious stimuli. The more recent discovery that both TRPV1 and TRPA1 are expressed on a multitude of non-neuronal sites has led to a plethora of research into possible functions of these receptors. Non-neuronal cells on which TRPV1 and TRPA1 are expressed vary from vascular smooth muscle to keratinocytes and endothelium. This review will discuss the expression, functionality and roles of these non-neuronal TRP channels away from sensory nerves to demonstrate the diverse nature of TRPV1 and TRPA1 in addition to a direct role in pain and neurogenic inflammation.

Abbreviations

PBMC, peripheral blood mononuclear cell; TRPA1, transient receptor potential cation channel subfamily A member 1; TRPV1, transient receptor potential cation channel subfamily V member 1

Introduction

The transient receptor potential vanilloid 1 and ankyrin 1 (TRPV1 and TRPA1, respectively) channels are members of the TRP superfamily of structurally related, non-selective cation channels that is divided into seven families: TRPC (Canonical), TRPN (no mechanoreceptor potential C), TRPM (Melastatin), TRPML (Mucolipin), TRPP (Polycystin), TRPA (Ankyrin) and TRPV (Vanilloid), each present in several species across the animal kingdom (Pedersen *et al.*, 2005; Clapham, 2007). TRP channels have many different physiological roles, ranging from purported roles as store-operated calcium channels (e.g. TRPC3, TRPC7; Riccio *et al.*, 2002; Kaznacheyeva *et al.*, 2007), to roles in thermo- (e.g. A1, M8, V1, V4; Caterina *et al.*, 1997; Güler *et al.*, 2002; Peier *et al.*, 2002; Story *et al.*, 2003), mechano- (e.g. A1, C1, V1, V4; Liedtke *et al.*, 2003; Walker *et al.*, 2003; Corey *et al.*, 2004; Maroto *et al.*, 2005) and chemo-sensation (e.g. A1, M8, V1; Peier *et al.*, 2002; Bandell *et al.*, 2004; Andersson *et al.*, 2008). All TRP channels are tetramers formed by subunits with six transmembrane domains and cation-selective pores, which frequently show high calcium permeability (Latorre *et al.*, 2009).

Whilst all seven families have wide roles in many physiological and pathophysiological processes, TRPV1 and TRPA1 will be the specific focus of this review. Both TRPV1 and TRPA1 play an integral role in pain (Bevan and Andersson, 2009; Fernandes *et al.*, 2011) and neurogenic inflammation (Geppetti *et al.*, 2008) via sensory nerve activation. In fact, 97% of TRPA1-expressing sensory neurons express TRPV1, while 30% of TRPV1-expressing neurons express TRPA1 (Story *et al.*, 2003). Both TRPV1 and TRPA1 channels are calcium-permeable, and, although their subunits have not been shown to form a heterotetramer channel, they may form a complex on the plasma membrane of sensory neurons. This enables TRPV1 to influence intrinsic characteristics of the TRPA1 channel, including voltage–current relationships and its open probability at negative holding potentials (Staruschenko *et al.*, 2010). Similarly, Salas *et al.* (2009) have shown that the features of

neuronal TRPA1 are not duplicated in cells expressing only TRPA1 and, instead, can be restored only when TRPA1 and TRPV1 channels are coexpressed. Moreover, both TRPV1 and TRPA1 are integrators of a range of noxious stimuli, and TRPV1 and TRPA1 agonists are able, at least in part, to heterologously desensitize TRPV1 and TRPA1 pathways (Ruparel *et al.*, 2008). Overall, based on evidence such as that described above, it may well be that TRPV1 and TRPA1 are 'partners in crime' in the activation of sensory nerves.

The role of these TRP channels in pain and neurogenic inflammation have, to date, been very well covered by previous authors (e.g. Bevan and Andersson, 2009; Cortright and Szallasi, 2009; Stucky *et al.*, 2009; Fernandes *et al.*, 2011), which reflects the enormity of the role that these channels play in sensory nerve function at both a central and peripheral level. However, there is accumulating evidence that TRPV1 and TRPA1 have functional roles away from sensory nerve activity, which this review aims to address. In order to substantiate a role for these non-sensory nerve TRP channels in either normal physiology or disease, several criteria must be fulfilled: (1) TRPV1/TRPA1 expression should be demonstrated in these tissues at the gene and protein level; (2) the receptors should be functional, that is, showing evidence of calcium permeability; and (3) a role in either physiological or pathophysiological functions should be demonstrated. In discussing these other functions, the imperative importance of TRPV1 and TRPA1 in pain and neurogenic inflammation cannot and should not be diminished. Furthermore, it is entirely possible that the TRPV1 and TRPA1 activity away from sensory nerves may indirectly affect pain and neurogenic inflammation.

TRPV1

The vanilloid TRP channels are divided into six members – 1 to 6. However, only TRPV1 from the TRPV subfamily is actually activated by vanilloids, including capsaicin, the pungent component of chilli peppers. The capacity of capsaicin to activate sensory nerves was determined by Jancsó in the 1960s (Jancsó *et al.*, 1967). However, it was 15 years later that the presence of a capsaicin receptor in the plasma membrane of sensory nerves was recognized by Szolcsányi and Jancsó-Gábor (1975). In 1997 came perhaps the most significant advance in TRPV1 research, when the mouse TRPV1 receptor was cloned by Caterina *et al.* (1997) [later cloned in humans (Hayes *et al.*, 2000) and guinea-pigs (Savidge *et al.*, 2002)]. With this major advance in the understanding of TRPV1 at a molecular level came the confirmation that heat >43°C and pH <5.9 also activate TRPV1 (Tominaga *et al.*, 1998). Furthermore, the generation of TRPV1 knockout mice and a comprehensive analysis of the phenotype of the animals confirmed a pivotal role for this receptor in noxious heat sensation *in vivo* (Caterina *et al.*, 2000). A variety of exogenous and endogenous activators of TRPV1 have since been identified (see Table 1).

In this context, it is important to mention that the TRPV1 receptor undergoes desensitisation after repeated administration of, or prolonged exposure to, capsaicin (Szolcsányi *et al.*, 1975) or resinaferatoxin (Szolcsányi *et al.*, 1990). The receptor

Table 1

Agonists and modulators

	TRPV1	TRPA1
Exogenous agonists	Capsaicin (Caterina *et al.*, 1997)	Allyl Isothiocyanate (Bandell *et al.*, 2004)
	Vanilloids (e.g. Olvanil, Resiniferatoxin) (Brand *et al.*, 1987; Szallasi and Blumberg, 1989)	Environmental pollutants, e.g. acrolein (Bautista *et al.*, 2006)
	Capsinoids (e.g. Capsiate) (Ohnuki *et al.*, 2001)	Irritants (e.g. Formalin) (Mcnamara *et al.*, 2007)
	Noxious high temperature (>43°C) (Caterina *et al.*, 1997)	Cold temperatures (<17°C) (Kwan *et al.*, 2006; Karashima *et al.*, 2009)
	Low pH (<6.0) (Caterina *et al.*, 1997)	Allicin (Macpherson *et al.*, 2005)
	Camphor (Xu *et al.*, 2005)	Icilin (Story *et al.*, 2003)
		Cinnamaldehyde (Macpherson *et al.*, 2006)
		Tetrahydrocannabinol (Jordt *et al.*, 2004)
Endogenous agonists	Anandamide (Zygmunt *et al.*, 1999; Smart *et al.*, 2000)	Oxidative stress products (Bessac *et al.*, 2008) e.g. 4-hydroxynonenal (Trevisani *et al.*, 2007).
	Lipoxygenase products (e.g. LTB4) (Hwang *et al.*, 2000; Huang *et al.*, 2002)	Lipid peroxidation products (Taylor-Clark *et al.*, 2008)
	N-acyldopamines (Huang *et al.*, 2002; Chu *et al.*, 2003)	Zinc, copper and cadmium (Hu *et al.*, 2009; Gu and Lin, 2010)
Endogenous modulators (via activation of intracellular pathways)	Bradykinin (Chuang *et al.*, 2001)	Bradykinin (Bandell *et al.*, 2004; S. Wang *et al.*, 2008)
	PAR-2 agonists (Amadesi *et al.*, 2004)	PAR-2 agonists (Dai *et al.*, 2007)
	NGF (Chuang *et al.*, 2001)	
	ATP (Chuang *et al.*, 2001)	

not only becomes desensitized to other activators of the same receptor, but the TRPV1 pathway heterologously desensitizes responses to TRPA1 agonists and vice versa (Ruparel *et al.*, 2008). The mechanisms underlying homologous and heterologous desensitization appear to be distinct with capsaicin-induced desensitization being calcium-dependent but heterologous desensitization being calcium-independent (Ruparel *et al.*, 2008). In addition, high doses of capsaicin selectively destroy C- and Aδ sensory nerves, thereby completely preventing nerve activation (Szallasi *et al.*, 1995). Clearly, this means that capsaicin-induced desensitization/nerve destruction cannot be used to examine TRPV1-specific down-stream effects on physiological/pathophysiological processes. However, it can be used to show that capsaicin-induced desensitization via TRPV1 has a functional effect upon a cell or tissue.

TRPA1

Although TRPA1 was identified slightly later than its TRPV1 counterpart, its discovery took a comparable course. The effects of the classic TRPA1 agonist, mustard oil (the active component being allyl isothiocyanate), were identified much earlier (e.g. Koltzenburg and McMahon, 1986) than the receptor itself, with this compound commonly being used to induce neurogenic inflammation, in a similar manner to capsaicin. TRPA1 was first cloned by Jaquemar *et al.* (1999), although its expression on neurons was not reported until 2003 when Story *et al.* identified it as a receptor for noxious cold temperature. However, the association between TRPA1 and mustard oil was later confirmed in 2005 when Jordt and colleagues not only established TRPA1 as the mustard oil receptor but also for tetrahydrocannabinol, leading the way into a plethora of research into further possible TRPA1 agonists (see Table 1). Indeed, concomitant with the groundbreaking generation of TRPA1 knockout mice (Bautista *et al.*, 2006) came the finding that TRPA1 mediates the effects of acrolein (2-propenal), present in tear gas, vehicle exhaust, tobacco products and byproducts of chemotherapeutic agents. Unlike the situation with TRPV1 knockout mice, an examination of the phenotype of TRPA1 knockouts regarding a role for this receptor in noxious temperature sensation has proved controversial. Two independent knockout animals have been generated (Bautista *et al.*, 2006; Kwan *et al.*, 2006). The portion of the gene knocked out was the fifth and sixth transmembrane domains (required for ion conduction) in both cases. However, Bautista *et al.* (2006) reported normal responses to cold temperatures both *in vivo* and *in vitro* in TRPA1 knockout mice. On the other hand, knockouts in the Kwan *et al.* (2006) study displayed a clear functional deficit to cold stimulation. A discussion of the reasons underlying these differences is beyond the scope of this review, but it remains to be seen whether TRPA1 is truly a thermosensor channel.

TRPV1 and TRPA1 expression

Central expression

The location of TRPV1 on small-diameter, Aδ and C fibre sensory nerves is clearly intrinsically linked with its role in pain and neurogenic inflammation. These nerves were the site of discovery for both TRPV1 and TRPA1 and are still today the focus of the bulk of research on these TRP channels. With sensory nerves, the effects of TRPV1 (and to a certain extent TRPA1) activation were known well before the receptor or its expression was identified. Effects of capsaicin in other cell types were also postulated as early as the 1970s (e.g. Jancsô and Wollemann, 1977). However, since the cloning of the receptors and the revolution of molecular biology, TRPV1 and TRPA1 expression on other cell types has been confirmed as an essential basis of the pharmacology of these effects. For example, Mezey *et al.* (2000) used a TRPV1-specific antibody to demonstrate the presence of TRPV1-expressing neurons throughout the neuroaxis, including such areas as the dopaminergic neurones of the substantia nigra, hippocampal pyramidal neurones, hypothalamic neurones and neurones in the locus coeruleus, in addition to various layers of the cortex. RT-PCR confirmed the expression of TRPV1 mRNA in the hippocampus, hypothalamus and cortex (Mezey *et al.*, 2000). The presence of TRPV1 mRNA has also been identified in the cerebellum (Sasamura *et al.*, 1998), showing a widespread of expression of TRPV1 within the CNS. However, more recently, the use of TRPV1 reporter mice has revolutionized the study of TRPV1 expression, and they would suggest that the expression of this receptor is minimal within a few discrete brain regions, most obviously in the vicinity of the caudal hypothalamus (Cavanaugh *et al.*, 2011).

The central localization of TRPA1 has been less specific than for TRPV1, although TRPA1 mRNA has been shown to be abundant in dog brain and cerebellum (Doihara *et al.*, 2009). However, associated protein expression was not confirmed in this study.

Peripheral expression

Within the periphery, recent evidence has located TRPV1 and TRPA1 on a variety of non-neuronal tissues. In fact, the list of possible sites of expression is becoming so great that to discuss all in turn would be outside of the scope of this article. Tables 2 and 3 list the peripheral non-neuronal cells on which TRPV1 and TRPA1 have been located by RT-PCR for RNA expression and/or immunohistochemical staining Western blot for protein expression. From this extensive list, it is clear to see that the advent of molecular biology techniques in this field a decade ago has revolutionized our potential understanding of the possible functions of these TRP channels. However, as mentioned previously, evidence that receptor mRNA or protein is present in a tissue should be substantiated by evidence that the channel is functional. Therefore, the following section will address that studies that have provided evidence of TRPV1/TRPA1 functionality in brain or peripheral sites.

Receptor functionality

Although TRPV1/TRPA1 channel expression has been shown in a wide variety of tissues, evidence of functionality has not yet been demonstrated for all of these. Therefore, this section will solely concentrate on cell types where both a molecular and functional presence has been confirmed. One of the first

Table 2

TRPV1 expression

Expression site	Immunostaining/ immunofluorescence)	RT-PCR	Western blot	[Ca^{2+}] functionality	Possible role/effects on activation	References
			TRPV1			
Mouse						
Arteriolar smooth muscle cells		✓		✓	Vasoconstriction	Cavanaugh *et al.*, 2011
Mesenteric arteries and endothelial cells	✓	✓		✓	Vasorelaxation	Yang *et al.*, 2010
Laryngeal epithelium	✓				Laryngeal nociceptors	Hamamoto *et al.*, 2008 (mouse); Hamamoto *et al.*, 2009 (human)
Preadipocytes and adipose tissue	✓	✓		✓	Adipogenesis	Zhang *et al.*, 2007b
Urothelium		✓			Stretch-evoked ATP release	Birder *et al.*, 2001
Rat						
Vascular smooth muscle	✓	✓			Vasoconstriction	Kark *et al.*, 2008
Pulmonary artery smooth muscle		✓	✓		Vasoconstriction	Yang *et al.*, 2006
Pancreatic B cells		✓	✓		Increased insulin secretion	Akiba *et al.*, 2004
Human						
Corneal epithelium			✓	✓	Inflammatory mediator secretion	Zhang *et al.*, 2007a
Corneal endothelium		✓	✓		Temperature sensation	Mergler *et al.*, 2010
Cerebromicrovascular endothelium	✓	✓		✓	Regulation of blood brain barrier permeability	Golech *et al.*, 2004
Blood	✓				Nociception; role in inflammatory processes?	Saunders *et al.*, 2007
Mononuclear cells		✓				
Epidermal keratinocytes	✓	✓		✓	Noxious chemical sensor	Inoue *et al.*, 2002
Preadipocytes and adipose tissue	✓	✓		✓	Adipogenesis	Zhang *et al.*, 2007b
Synoviocytes		✓		✓	Adaptive/pathological changes in arthritic inflammation	Kochukov *et al.*, 2006
Nasal vascular endothelium, epithelials and submucosal glands	✓	✓			Stimulate epithelial secretions	Seki *et al.*, 2006

Table 3

TRPA1 expression

Expression site	Immunostaining/ immunofluorescence)	RT-PCR	Western blot TRPA1	$[Ca^{2+}]$ functionality	Possible role/effects on activation	References
Mouse						
Auditory hair cell; organ of corti; utricle, saccule and crista ampullaris	✓		✓	✓	Mechanosensor?	Nagata *et al.*, 2005
Enterochromaffin cells		✓		✓	Regulates gastrointestinal motility via 5HT release	Nozawa *et al.*, 2009
Hair follicle keratinocytes	✓	✓			Modulation of cutaneous nerve firing	Kwan *et al.*, 2009
Rat						
Cerebral and cerebellar artery endothelium	✓	✓		✓	Vasodilation	Earley *et al.*, 2009
Urothelium	✓	✓			Detrusor overactivity	Streng *et al.*, 2008
Enterochromaffin cells		✓		✓	Regulates gastrointestinal motility via 5HT release	Nozawa *et al.*, 2009
Human						
Undifferentiated keratinocytes				✓	Thermosensation??	Tsutsumi *et al.*, 2010
Skin basal keratinocytes	✓				?	Anand *et al.*, 2008
Keratinocytes in epidermis and dermis of hair follicle		✓	✓		Keratinocyte differentiation; inflammation; mechano- and thermosensor	Atoyan *et al.*, 2009
Melanocytes		✓	✓			
Fibroblasts		✓	✓			
Synoviocytes		✓		✓	Adaptive/pathological changes in arthritic inflammation	Kochukov *et al.*, 2006
Enterochromaffin cells		✓		✓	Regulates gastrointestinal motility via 5HT release	Nozawa *et al.*, 2009
Other						
Dog brain and cerebellum		✓			?	Doihara *et al.*, 2009

cell types in which functionality was first identified is epidermal keratinocytes. Inoue *et al*. (2002) demonstrated that both capsaicin and acidification produced elevations in the intracellular calcium concentration in cultured human epidermal keratinocytes. Furthermore, these increases were inhibited by the TRPV1 antagonist, capsazepine (Inoue *et al*., 2002). Similarly, treatment of human skin fibroblasts with capsaicin induced significant changes in the membrane current and the intracellular calcium level that were antagonized by capsazepine (Kim *et al*., 2006). More recently, TRPA1 agonists have also been shown to activate calcium currents in both keratinocytes (cold, allyl isothiocyanate and mustard oil; Atoyan *et al*., 2009) and fibroblasts (cold, allyl isothiocyanate and cinnemaldehyde; Hu *et al*., 2010). This trend is carried forth with a multitude of other cells, as summarized in Table 2.

It is therefore clear to this point that TRPV1 and TRPA1 are expressed and functional away from sensory nerves. Nevertheless, this does not mean that the channels have similar sensitivities. However, a comparison of the sensitivity of channels expressed on sensory nerves and other tissues regarding agonist stimulation is extremely difficult to extract from the literature. The only study that to date has directly compared neuronal and non-neuronal TRPV1 responses is that by Kark *et al*. (2008) in vascular tissue. In the vasculature, capsaicin has biphasic effects: at lower concentrations up to 10 nM, dilations are observed in response to neuronal TRPV1 activation. Conversely, at higher capsaicin concentrations between 0.1 and 1 μM, vasoconstriction is observed in response to non-neuronal TRPV1 activation.

In other cell types, capsaicin increased the intracellular calcium concentration of rat vagal neurons within a capsaicin concentration range of 0.1–10 μM (Marsh *et al*., 1987) and increased the intracellular calcium concentration of human and murine adipocytes within a capsaicin concentration range of 0.01–1 μM (Zhang *et al*., 2007). The sensitivity of neuronal and non-neuronal TRPV1 to capsaicin activation in these studies therefore looks similar. However, it is impossible to directly compare studies due to differences in experimental conditions, including species differences (rat vs. mouse/human, respectively, for the Marsh and Zhang studies). Thus, the study by Kark *et al*. (2008) provides the only evidence to date that there may be differences in sensitivity between neuronal and non-neuronal forms of the receptor. The sensitivity of TRPA1 on sensory nerves compared with TRPA1 on other tissues is also very difficult to discuss, as no study has directly compared the same agonist in each type of tissue across a concentration range. Thus, any difference in sensitivity remains to be seen.

Role in physiological or pathophysiological function

From the previous sections, it is clear that the range of cell types expressing functional TRPV1 and TRPA1 is very diverse. As such, it is not surprising that the associated functions of these receptors are also complex. Physiological or pathophysiological effects of non-neuronal TRPV1 and TRPA1 have been implicated in inflammation, infection and immunity, the cardiovascular system and in conditions such as obesity. Meanwhile, neuronal TRPV1 in the brain may have functions in neurogenesis (Jin *et al*., 2004) and thermoregulation (Jancsó-Gábor *et al*., 1970b), amongst others. The following sections will discuss the effects of TRPV1 and TRPA1 in the brain as well as non-neuronal TRPV1 and TRPA1 within the context of different physiological/pathophysiological systems. In doing so, the possible significance of these TRP channels away from sensory nerves will be highlighted.

In the brain

As mentioned previously, TRPV1 is expressed throughout the brain and, moreover, these TRPV1 channels appear to be functional upon agonist stimulation. Indeed, downstream of channel activation, Jancsô and Wollemann (1977) have reported that capsaicin stimulates adenylate cyclase activity in the rat cerebral cortex *in vitro*. Furthermore, direct injection of capsaicin into the preoptic area of the anterior hypothalamus (Jancsó-Gábor *et al*., 1970b) or i.c.v. region (Dib, 1982) of the rat brain causes hypothermia, suggesting a role for this channel in thermoregulation. Hypothermia is associated with a fall in rectal and hypothalamic temperature, an increased cutaneous temperature (Dib, 1982) and tail skin vasodilation (Jancsó-Gábor *et al*., 1970a). Vice versa, rats desensitized by hypothalamic injections of high concentrations of capsaicin lose their ability to thermoregulate against overheating of their bodies and respond with an enhanced hyperthermia to strong sensory stimuli such as repeated pinching of the tail (Jancsó-Gábor *et al*., 1970a). Similarly, systemic administration of TRPV1 antagonists such as AMG517 (Gavva *et al*., 2007b), AMG0347 (Steiner *et al*., 2007) and A-425619 (Gavva *et al*., 2007a) causes an increase in body temperature within approximately 1 h of treatment. However, antagonist-induced hyperthermia may not be mediated by hypothalamic TRPV1 as peripherally-restricted antagonists still have the capacity to cause an increase in body temperature (Tamayo *et al*., 2008).

In inflammation, infection and immunity

A physiological/pathophysiological role for non-neuronal TRPV1/TRPA1 is perhaps nowhere more apparent than in the case of inflammation, infection and immunity. However, it is important to note that although the effects of these TRP channels are non-neuronal, it is evident that they may well impact indirectly upon pain and/or neurogenic inflammation. As mentioned previously, keratinocytes functionally express both TRPV1 and TRPA1. These cells play an important role in maintaining the integrity of the immune response in skin as well as stimulating cutaneous inflammation via prostanoid and cytokine release (Gröne *et al*., 2002). TRPV1 activation by capsaicin causes an increase in COX2 expression in human keratinocytes with a concomitant increase in PGE_2 levels *in vitro* (Southall *et al*., 2003). An increase in IL-8 is also observed (Southall *et al*., 2003). Similarly, treatment of human keratinocytes with the TRPA1 agonist, icilin, has been

shown to increase the expression of pro-inflammatory ILs (IL-1α and IL-1β; Atoyan *et al.*, 2009) as well altering the expression of genes involved in the control of keratinocyte proliferation, differentiation and cell cycle regulation (Atoyan *et al.*, 2009). Stimulation of inflammatory mediator release by TRPV1/TRPA1 agonists from keratinocytes could well have a significant effect upon sensory nerves that have a high density in skin, especially as PGE_2 and IL-1 are known to sensitize and/or activate sensory nerve endings (Schaible and Schmidt, 1988; Binshtok *et al.*, 2008). This is therefore a prime example of how non-neuronal TRPV1/TRPA1 may interact with sensory nerves to affect pain and neurogenic inflammation.

As well as keratinocytes, peripheral blood mononuclear cells (PBMCs) are also directly affected by TRPV1/TRPA1 activation. For example, PBMCs undergo apoptosis when stimulated with capsaicin or resinaferatoxin, an effect that is reversed by the TRPV1 antagonist, AM630 (Saunders *et al.*, 2007). In relation to TRPA1, the cinnamaldehyde derivative, 2′-hydroxycinnamaldehyde has been shown to inhibit nitric oxide release and NF-κB activation in macrophages that have been stimulated with LPS (the cell wall component of Gram-negative bacteria; Lee *et al.*, 2005). Similarly, cinnemaldehyde inhibits IL-1β and TNFα release from human monocytes and macrophages that have been stimulated by LPS (Chao *et al.*, 2008). A concomitant reduction of the release of reactive oxygen species from the macrophages is also observed (Chao *et al.*, 2008). It would therefore appear that the effects of TRPV1 and TRPA1 in terms of their direct effects on inflammatory cells appear to be in part anti-inflammatory.

There are various other cells involved in immunity that respond to TRPV1 and TRPA1 activation including bone marrow-derived dendritic cells, where capsaicin leads to dendritic cell maturation and an increase in antigen presentation (Basu and Srivastava, 2005). Furthermore, with regard to TRPA1, cinnamaldehyde has been shown to cause a dose-dependent suppression of the lymphoproliferation in LPS-treated mouse splenocytes (Koh *et al.*, 1998). The same study also showed that the exposure of thymocytes to cinnamaldehyde accelerated T-cell differentiation from CD4 and CD8 double-positive cells to CD4 or CD8 single-positive cells (Koh *et al.*, 1998).

It is therefore clear that TRPV1 and TRPA1 are expressed, functional and are active within cells relevant to inflammation, infection and immunity. Most of the aforementioned studies have been carried out *in vitro* and so the precise influences of these effects in an *in vivo* setting are, as yet, far from clear. However, what it is clear from other studies is that TRPV1 at least plays a paradoxical role in inflammation *in vivo*, for example, exacerbating inflammation in arthritis and yet in experimentally induced sepsis, TRPV1 null mice demonstrate elevated levels of pathological markers in comparison with wild-type mice (Alawi and Keeble, 2010). It cannot be ruled out at this stage that this is due to differing effects of neuronal and non-neuronal TRPV1 channels.

Role in the vasculature

TRPV1 and TRPA1 have been shown to control vascular responses either by the well-established neurogenic response that is mediated by sensory nerves (Geppetti *et al.*, 2008) or via a direct effect on vascular tissue (Kark *et al.*, 2008; Earley *et al.*, 2009). However, the non-neuronal mechanisms involved in mediating vasodilatation and oedema formation following TRPV1 and TRPA1 activation *in vivo* are unclear. Both endothelial cells and smooth muscle cells express a variety of membrane ion channels to control Ca^{2+} influx and membrane potential, including the expression of TRPV1 and TRPA1 channels on endothelial cells (Yao and Garland, 2005; Earley *et al.*, 2009) and TRPV1 expression on vascular smooth muscle cells (Kark *et al.*, 2008; Cavanaugh *et al.*, 2011), as described in Table 1. Thus, there is clearly the potential for non-neuronal TRPV1 and TRPA1 to contribute to vasculature control.

TRPV1 on endothelial cells has been shown to regulate the expression and secretion of endothelial cell-derived CGRP, which affords protective effects on endothelial cells (Luo *et al.*, 2008). Furthermore, CGRP is a potent vasodilator (Brain *et al.*, 1985), and this CGRP may therefore impact upon blood pressure. Indeed, TRPV1 activation on sensory nerves also causes CGRP release, leading to a profound decrease in vascular tone (Zygmunt *et al.*, 1999). On the other hand, TRPV1 expressed on vascular smooth muscle appears to cause vasoconstriction. Kark *et al.* (2008) have shown that capsaicin triggers transient vasoconstriction in isolated pressurised rat skeletal muscle arterioles, which is not abolished by endothelial cell removal or denervation *in vivo*, indicating the vasoconstriction was mediated by a direct effect of TRPV1 on vascular smooth muscle. Keeble and Brain (2006) have also demonstrated vasoconstrictor responses to capsaicin, albeit in the mouse synovial membrane. More recently, Cavanaugh *et al.* (2011) have demonstrated vasoconstriction in response to capsaicin in mouse ear arterioles. Interestingly, as mentioned previously, it has also been suggested that capsaicin has biphasic effects on the vasculature: at lower concentrations, capsaicin (up to 10 nM) evokes vasodilation in skin due to sensory nerve activation, whereas higher concentrations (0.1–1 μM) elicit substantial constrictions in skeletal muscle arterioles due to non-neuronal TRPV1 stimulation (Kark *et al.*, 2008). It is unclear whether this difference is due to receptor sensitivity (as discussed earlier with respect to receptor functionality) or a difference in TRPV1 receptor density in the two tissues. Furthermore, it is not entirely clear whether, in order to achieve vasoconstriction, the vasodilator effect of capsaicin first needs to be counteracted. However, it is possible that highly localised TRPV1 activation by endogenous activators means that vasoconstriction or vasodilation are triggered entirely separately, as opposed to treatment with exogenous capsaicin when all TRPV1 is likely to be affected simultaneously.

TRPV1 may also play a role in vascular responses during chronic hypoxia where up-regulation of the TRPV1 gene and protein is observed (Y.X. Wang *et al.*, 2008). Chronic hypoxia has been shown to enhance the ability of human pulmonary artery smooth muscle cells to proliferate and to increase resting levels of cytosolic calcium and capacitative calcium entry with both effects being inhibited in a dose-dependent manner by the TRPV1 antagonist, capsazepine (Y.X. Wang *et al.*, 2008). These results therefore suggest that TRPV1 on smooth muscle may be a critical pathway or mediator in chronic hypoxia-induced vascular changes.

Research into TRPA1 in the vasculature is still in an early phase although mustard oil has been shown to trigger vasodilatation in rat cerebral arteries via a mechanism that appears to involve TRPA1 expressed on endothelial cells (Earley *et al.*, 2009). Mustard oil-induced vasodilation was not mediated by nitric oxide or prostanoids, rather by calcium-activated potassium channels on endothelial cells and inwardly rectifying potassium channels on arterial myocytes. Furthermore, the responses were inhibited by the TRPA1 antagonist, HC-030031 (Earley *et al.*, 2009).

It is therefore clear that non-neuronal TRPV1 and TRPA1 both have the potential to play a role in the physiology or pathophysiology of the vasculature. TRPV1 in general has been shown to play a role in hypertension (Li and Wang, 2003), cardiac ischaemia (Wang and Wang, 2005) and cardiovascular shock (Akabori *et al.*, 2007). However, the relative contribution of neuronal and non-neuronal TRPV1 to these effects is, as yet, far from clear. In the case of TRPA1, the physiological relevance of TRPA1 on sensory nerves in the vasculature has only just been elucidated (Pozsgai *et al.*, 2010), and so we still have a long way to go with our understanding of this channel.

Obesity and thermogenesis

Obesity is one of the most significant health issues in western society due to the morbidity associated with this condition that is increasing in prevalence. Thus, a significant amount of research has been generated to understand its underlying causes and means of treating/preventing the condition. It is known that obesity is induced by the hypertrophy of adipocytes and the recruitment of new adipocytes from precursor cells. These processes are dependent on the regulation of adipocyte differentiation. The TRPV1 receptor is very interesting in this respect as capsaicin has been shown to inhibit adipocyte differentiation *in vitro* by activation of AMP-activated protein kinase (Hwang *et al.*, 2005). Furthermore, Hsu and Yen (2007) have shown that treatment of preadipocytes with capsaicin decreases the number of normal adipocytes and increases the number of early apoptotic and late apoptotic cells in a dose-dependent manner. Furthermore, treatment of adipocytes with capsaicin was shown to decrease the quantity of intracellular triglycerides and glycerol-3-phosphate dehydrogenase activity (Hsu and Yen, 2007), both biomarkers of adipogenesis.

As with the previous physiological/pathophysiological conditions discussed, it is not known how significantly non-neuronal TRPV1 receptors contribute to the overall effects of TRPV1 activation. However, the overall effect of TRPV1 modulation in obesity is stark. For example, both animal (Zhang *et al.*, 2007) and human (Ohnuki *et al.*, 2001) data have indicated that the consumption of capsaicin- or non-pungent capsiate-containing foods is correlated with a reduced incidence of obesity. Similarly, oral administration of capsaicin alone also suppresses body fat accumulation in mice (Ohnuki *et al.*, 2001), and dietary capsaicin can reduce obesity-induced insulin resistance and hepatic stenosis in mice fed a high fat diet (Kang *et al.*, 2010). Moreover, TRPV1-mediated changes in thermogenesis may have the potential to impact upon obesity, possibly through changes in expression of thermogenic uncoupling proteins, as seen in response to chronic treatment of rats with capsiates (Masuda *et al.*, 2003).

In recent years, a role for TRPV1 in thermoregulation has also been identified which may, at least in part, be due to changes in thermogenesis (for review, see Romanovsky *et al.*, 2009). For many years, capsaicin has been known to cause a centrally mediated hypothermia in mice (Jancsó-Gábor *et al.*, 1970b). In contrast, its intragastric administration enhances thermogenesis and heat diffusion (Masamoto *et al.*, 2009). Similarly, the jejunal administration of non-pungent capsaicin analogues was shown to increase energy expenditure via direct activation of TRPV1 located on intestinal extrinsic nerves (Kawabata *et al.*, 2009). Interestingly, some TRPV1 antagonists cause hyperthermia, associated with increased thermogenesis (Gavva *et al.*, 2007a) through a peripheral mechanism (Tamayo *et al.*, 2008), whilst TRPV1 gene knock down does not affect body temperature in mice (Tóth *et al.*, 2011). and TRPV1 knockout mice exhibit a normal basal body temperature (Steiner *et al.*, 2007). Although this clearly shows a homeostatic role for TRPV1 in thermoregulation, it is beyond the scope of this review to discuss the mechanism underlying TRPV1 antagonist-induced hyperthermia as there is, to date, no direct evidence that it is mediated by non-neuronal TRPV1. It will be extremely interesting in the future to determine whether the mechanism underlying role of TRPV1 in thermoregulation is intrinsically linked with the aforementioned role for TRPV1 in obesity.

Conclusion

To conclude, it is now clear that the roles of TRPV1 and TRPA1 discussed in this review extend far beyond sensory nerves. Not only are TRPV1 and TRPA1 receptors expressed in other neuronal and non-neuronal tissues, but they also exhibit functionality and they are of potential physiological/pathophysiological relevance. It is clear that we still have a great deal to learn about these receptors away from sensory nerves, especially in relation to their precise function *in vivo*. We also need to find out a great deal more about their influence upon pain and neurogenic inflammation as it is entirely possible that they are intrinsically related. Progress in this field would be greatly enhanced by selective knockout/knockdown of TRPV1/TRPA1 on sensory nerves and/or other specific cell types. Furthermore, it would be interesting to determine whether TRPV1 antagonists, or the currently available TRPA1 antagonists, have any relative specificity for these TRP channels on different cell types. Finally, it remains to be seen whether different modes of activation of TRPV1 and TRPA1 have potentially differing importance depending on the site of TRP channel expression. We eagerly await these answers.

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Conflict of interest

There is no known conflict of interest.

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